REMARKS

Reconsideration of this application is respectfully requested.

Claims 284-400 were previously pending in this application. Claims 284, 314, 315, 329, 331, 332, 337, 348, 373, 376, 377, 381, 385 and 390 have been amended above. Claims 401-512 have been added. No claims have been canceled by this Fourth Supplemental Amendment. Accordingly, claims 284-512 are presented for further examination on the merits.

This paper follows, of course, Applicants' Third Supplemental Amendment that was filed on March 29, 1999, about one week after the March 23. 1999 interview held among Examiner (Dr.) Marschel, Dr. Dean L. Engelhardt, Senior Vice President of Enzo Biochem, Inc. and Applicants' undersigned attorney of record. The remarks in this paper are intended to address the issues raised and discussed at the March 23rd interview.

As indicated above, claims 284, 314, 315, 329, 331, 332, 337, 348, 373, 376, 377, 381, 385 and 390 have been amended. In each of 284, 331, 337 and 348, the designation of SM has been changed from a "monosaccharide moiety" to the more limited term "furanose moiety." In claim 284, an inadvertent repetition for nucleotide (ii) has been corrected. That is to say, the language "wherein PM is a phosphate moiety, SM is a monosaccharide moiety, and BASE is a pyrimidine, purine or 7-deazapurine moiety" has been deleted in the second instance for nucleotide (ii). Furthermore, the first step (a) in claim 284 has been amended to recite "hybridizing said nucleic acid of interest in the sample with one or more oligo- or polynucleotides, each such oligo- or polynucleotide being complementary to or capable of hybridizing with said nucleic acid of interest or a portion thereof, . . ."

In addition to the change to "furanose" moiety in claim 284, for each of the recited nucleotides (i), (ii) and (iii), the attachment positions of the

phosphate moiety PM to the furanose moiety SM has been expressed as a Markush group. Thus, for each nucleotide, "PM is attached to the furanose moiety SM at a position selected from the group consisting of the 2', the 3' and the 5' position, or any combination thereof . . ."

Claims 314 and 315 have also been rendered into multiple dependent form in the amendments above. Both claims now depend from claims 312 or 313.

Claim 329 has been amended both in response to an issue raised at the March 23, 1999 interview and for the sake of clarity and preciseness. The incorporating language has been expunged in favor of a providing step. Thus, the first step of claim 329 now recites "providing labeled nucleic acid fragments, each fragment being complementary to a portion of or to said nucleic acid of interest, wherein each of said fragments comprise one or more modified nucleotides, said modified nucleotide or nucleotides being modified on the sugar, phosphate or base moieties thereof, and comprising detectable or self-indicating labels." The second step of claim 329 has been amended to recite "subjecting said labeled fragments to a sequencing gel to separate or resolve said fragments." Previously, the second step recited "separating said labeled nucleic acid or labeled nucleic acid fragments in a sequencing gel." Finally, the last step of claim 329 recites has been changed to read "detecting the presence of each of said separated or resolved fragments by means of said detectable or self-indicating labels, thereby determining the sequence of said nucleic acid of interest."

As indicated above, claim 331, which depends from claims 329 or 373, has been similarly amended as in the case of 284. That is to say, the SM element has been limited to a "furanose moiety" and the positions for the attachment of the phosphate PM to the furanose moiety SM have been recited in Markush language. The designation of SM as a "furanose moiety" has also been made to claim 332, which like claim 331, also depends from claims 329 or 373.

Substantial clarification has been made to claim 337 which is directed to a process for preparing a labeled oligo- or polynucleotide of interest. As in the case of the other claims, SM is now a "furanose moiety" and the phosphate PM is attached to the furanose moiety SM at the Markush positions previously discussed above in this paper. In addition, the first step (A) has been changed to recite in the alternative, providing either "(1) one or more chemically modified nucleotides capable of incorporating into an oligo- or polynucleotide of interest, . . .; or (2) an oligo or polynucleotide of interest comprising one or more chemically modified nucleotides, alone or in conjunction with one or more other modified or unmodified nucleic acids selected from the group consisting of nucleotides, oligonucleotides and polynucleotides, . . ." The second step (B) in claim 337 has also been changed to conform with the new language in step (A). As amended above, step (B) in claim 337 now reads "either incorporating said one or more modified nucleotides (1) into said oligo- or polynucleotide, thereby preparing a labeled oligo- or polynucleotide of interest, or preparing said oligo- or polynucleotide of interest from said oligo- or polynucleotide (2)."

The changes to claim 348 include the following. First, the preamble of the claim has been changed to read "[a] process for determining in a sequencing gel the presence of nucleic acid fragments complementary to a nucleic acid of interest or a portion thereof . . ." Further, the phrase "or forming one or more nucleic acid fragments, each such fragment being complementary to said nucleic acid of interest or to a portion thereof" has been inserted into the first providing step (A) of the claim. Also changed in claim 348 is the substitution of "nucleic acids or nucleic acid fragments" in place of "oligo- or polynucleotide." With the amendments above, the second step (B) in claim 348 reads "incorporating said one or more chemically modified nucleotides into said one or more fragments, thereby preparing labeled fragments, each such fragment being complementary to said nucleic acid of interest or to a portion thereof, said labeled fragments comprising one or more chemically modified nucleotides selected from . . ." Changes to the Markush group of nucleotides (i), (ii) and (iii) in claim 348 include the previously discussed "furanose" moiety and the recitation of Markush positions for the attachment of the phosphate moiety PM to the furanose moiety SM. Finally, relatively minor

amendments have been made to the last three steps of claim 348. These steps read as follows: "(C) transferring or subjecting said labeled fragments to a sequencing gel; (D) separating or resolving said labeled fragments; and (E) detecting directly or indirectly the presence of said labeled fragments."

In claim 373, a sequencing process as in the case of claim 329, Applicants have effected minor changes to each of the four process steps. As amended above, the first step of claim 373 recites "providing or generating labeled nucleic acid fragments complementary to said nucleic acid of interest or to a portion thereof, each of said labeled fragments comprising one or more modified nucleotides which comprise detectable or self-indicating labels and said one or more modified nucleotides being modified on the sugar, phosphate or base moieties thereof." The second step recites "introducing or subjecting said fragments to a sequencing gel." This is followed by the steps of "separating or resolving said fragments in said sequencing gel" and "detecting each of the separated or resolved fragments; thereby determining the sequence of said nucleic acid of interest."

In the process claims for chromosomal characterization, parallel amendments have been made above as follows. First, in claim 376 which is independent, the oligo- or polynucleotide recited therein has been pluralized and further defined as being "capable of hybridizing specifically to a locus or loci of said particular chromosome or a portion thereof." Second, as discussed above in the other claims, the attachment positions of the phosphate moiety PM to the furanose moiety SM has been expressed in Markush language. Finally, reference to "the locus or loci" of said particular chromosome has been made in the phrase "to permit hybridization of said oligo- or polynucleotide . . ." The elements in claim 377 which depends from the aforementioned claim 376 have also been pluralized. Thus, as amended, claim 377 reads "wherein said one or more oligo- or polynucleotides comprise a clone or clones or DNA fragments derived from said particular chromosome."

The changes described above in claim 376 with respect to the oligo- or polynucleotide having been pluralized, the attachment positions of PM to

SM being recited in Markush format, and reference to a "locus or loci" in a chromosome, have also been implemented in claims 381, 385 and 390.

None of the foregoing amendments to the claims is believed to constitute the insertion of new matter into Applicants' original disclosure.

In new claims 401-512, Applicants are claiming dependent embodiments, nearly all of which are mirrored in the former dependent claims or the present dependent claims. To begin with, new claims 401-403 and 511-512 relate to the chromosomal claims. Claims 401 and 402 define the oligo- or polynucleotide as being "labeled with the same or a different indicator molecule." Claim 403 mimics claim 377 by reciting the process of claims 381, 385 or 390, "wherein said one or more oligo- or polynucleotides comprise a clone or clones or DNA fragments derived from said particular chromosome." Finally, in claims 511 and 512, embodiments of the detectable moiety Sig have been recited in Markush format.

With the exception of claim 510, the rest of the newly added claims further limit the independent sequencing claims, 329, 348 and 373, by limiting or defining the detectable moiety or moieties. That lone exception, claim 510, limits the detection process of claim 284 by reciting the inclusion of "one or more washing steps." The rest of the claims, 404-509 can be summarized as follows:

Claim No(s).	Subject Matter/Embodiment
404-406	each of said nucleic acid fragments is labeled with the same or a different indicator molecule
407-409	said fragments have been obtained or generated by a nucleic acid sequencing step or technique
410-412	Sig or A (or Sig or A) comprises at least three carbon atoms

Claim No(s).	Subject Matter/Embodiment
413-415	Sig or A (or Sig or A) comprises a monosaccharide, polysaccharide or oligosaccharide
416-418	Markush members for Sig, A (or Sig or A)
419-421	electron dense component
422-424	ferritin
425-427	magnetic component
428-430	magnetic oxide or magnetic iron oxide
431-433	magnetic beads
434-436	sugar residue . . . complexed with or attached to a sugar binding protein or a polysaccharide binding protein
437-439	binding protein comprises a lectin
440-442	lectin comprises Concanavalin A
443-445	lectin is conjugated to ferritin
446-448	enzyme
449-451	Markush group of enzymes
452-454	hormone
455-457	radioactive isotope
458-460	metal-containing component
461-463	metal-containing component is catalytic
464-466	fluorescent component
467-469	Markush group of fluorescent components
470-472	chemiluminescent component

Claim No(s).	Subject Matter/Embodiment
473-475	antigenic or haptenic component capable of complexing with an antibody specific to the component
476-478	antibody component
479-481	chelating component
482-484	Sig or A (or Sig or A) is detectable when said modified nucleotides are contained in a double-stranded ribonucleic or deoxyribonucleic acid duplex
485-487	Sig or A (or Sig or A) is detectable when it is attached to the nucleotide directly or through a linkage group
488-490	linkage group does not interfere substantially with the characteristic ability of Sig or A (or Sig or A) to form a detectable signal
491-494	covalent attachment to PM via a chemical linkage
495-497	nucleic acid fragments are terminally ligated or attached to a polypeptide
498-500	polypeptide comprises polylysine
501-503	Markush members for the polypeptide
504-506	Sig or A (or Sig or A) comprises a ligand and the polypeptide comprises an antibody thereto
507-509	Markush members for a moiety which can be detected when a complex is formed between Sig or A (or Sig or A) and said polypeptide

None of the aforedescribed claims 401-512 is believed to constitute the insertion of new matter into the original disclosure. Entry of new claims 401-512 is respectfully urged.

Turning to other matters, the following six issues were discussed at the March 23, 1999 interview:

1. 5' terminal labeling of nucleic acids and oligo- or polynucleotides
2. self-indicating
3. disruptive base labeling positions
4. monosaccharide (since amended above to "furanose")
5. fragments language in claim 329
6. February 2, 1999 amendments to independent claims

1. **5' Terminal Labeling**

It is believed that the original disclosure fully describes and enables 5' terminal labeling of oligo- or polynucleotides. Several portions in the specification are dispositive on this issue.

As noted in earlier responses, Applicants disclose in Example V on page 57 of the specification a means for terminally labeling nucleic acids, including 5' terminal labeling. In Example V, Applicants disclose:

> ***Biotin and polybiotinylated poly-L-lysine were coupled to oligoribonucleotides using a carbodimide coupling procedure described by Halloran and Parker, J. Immunol., 96 373 (1996).*** As an example, DNA (1 µg/ml), 1 ml) in tris buffer pH 8.2, sheared with 0.1 N sodium hydroxide was denatured by boiling for 10 minutes and quick cooling in an ice bath. Biotinyl-1,6-diaminohexane amide (2 mg, 6 µmol) or polybiotinylated poly-L-lysine (2 mg) and 1-ethyl-3-diisopropylaminocarboimide HCl (10 mg, 64 µmol) were added,and the pH readjusted to 8.2. After 24 hours at room temperature in the dark, the mixture was dialyzed against 10 mM tris buffered saline. DNA was precipitated ethanol. [bold & italic added]

Although the first sentence above refers to "oligoribonucleotides," it is quite plain and can be easily gleaned from the rest of the quoted passage that Example 5 pertains to 5' terminal labeling of nucleic acids like DNA, and not just RNA. Using the procedure disclosed in Example 5, one obtains in part nucleic acid in the form of DNA wherein the 5' terminus is labeled with a biotinylated poly-L-lysine. This is also evidenced by Figure 2 in Halloran

and Parker's 1966 paper where the 5' position of the sugar is modified and depicted as the terminus of a deoxyribonucleotide. As described in Example V in Applicants' disclosure and as explained above, nucleic acid in the polymeric forms of DNA and RNA can also be modified in accordance with the present invention.

As another example of 5' terminal labeling, Applicants disclose on page 25, second paragraph:

> Also, the compounds can be prepared by terminal addition to oligo- or polynucleotides to produce compounds in which m or n is 0 depending on whether the addition is at the ***5'*** or 3' position. Moreover, the ***compounds such as pCp or pUp in which the base is biotinized can be added to existing molecules employing the enzyme RNA ligase***.
> [emphasis added]

The above-quoted portion is taken from U.S. Patent Application Serial No. 06/255,223, filed on April 17, 1981, which is exclusively licensed to the present Assignee. Dr. David C. Ward of Yale University, a recent inductee into the National Academy of Sciences, was a co-inventor on that application. Subsequent continuations or divisional applications of Serial No. 06/225,223 issued as U.S. Patent Nos. 4,911,755; 5,328,824; 5,449,767; and 5,476,928, each of which makes the same disclosure as in the present application.

Later on pages 77 and 78 of the present specification, Applicants provide an actual example that shows 5' labeling of a nucleic acid probe. This demonstration, memorialized in the form of Example 34, is set forth below:

> ***A DNA probe was ligated to a synthetic DNA composed of repeated sequences of E. coli lac operator DNA. After hybridization to detect antiprobe sequences, the hybridized DNA was detected by reaction with biotinylated lac repressor which was, in turn, detected by an enzyme linked immuno sorbent assay using goat antibiotin IGG to react with the biotin and a second antibody coupled to horse radish peroxidase.*** The lac polyoperator DNA has been described by Caruthers (Second Annual Congress for Recombinant DNA Research, Los Angeles, 1982), and it was ligated, in a blunt end ligation, using

> T4 ligase, to an adenovirus DNA probe. In situ hybridization of the polyoperator-labeled probe DNA was carried out as described by Gerhard et al (Proc. Natl. Acad. Sci. USA, 78, 3755 (1981). Biotinylated lac repressor was prepared as described by Manning et al (Chromosoma, 53, 107-117 (1075) and was applied to adenovirus infected cells, fixed to a glass slide, in Binding buffer composed of (0.01 MK Cl, 0.01 M tris (pH 7.6), 0.01 M $MgSO_4$, 10^{-4} MEDTA, 10^{-4} DTT, 5% DMSO (dimethyl sulfoxide) and 50 µg/ml bovine serum albumin by J. Miller, Experiments in Molecular Genetics, Cold Spring Harbor Laboratory (1972). The slides were washed in binding buffer to remove unbound biotinylated lac repressor and then assayed for biotin using the horse radish peroxidase-linked double antibody procedure. This procedure could be adapted to create an affinity column where the probe could be bound to immobilized repressor protein and then removed by elution with a specific inducer, for example, isopropylthigalactoside or thiomethylgalactoside. The affinity of the repressor- operator complex is quite high 10^{-11} M. When a specific inducer binds to the repressor the operator- repressor complex collapses.[1] [bold & italic added]

As disclosed in the above Example 34, when nucleic acid, in this instance, a DNA probe, is ligated, in this instance through blunt end ligation using T4 ligase, the labeled nucleic acid, in this instance, a synthetic DNA composed of repeated sequences of E. coli lac operator DNA, will attach to both the 5' and 3' ends of each strand of the probe DNA. Blunt end ligation was well known in the art, and resort could have been made to any number of sources, including, for example, Maniatis et al., Molecular Cloning: A Laboratory Manual (1982), who provide several protocols for carrying out blunt end ligation.

It is believed that the foregoing remarks, including the portions of the present specification quoted above, is sufficient to allay any concerns regarding either the written description of 5' terminal labeling of oligo- or polynucleotides or the enabling practice of 5' terminal labeling with chemically modified nucleotides in accordance with Applicants' invention.

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[1] **Although they were previously submitted in one or more Information Disclosure Statements submitted in connection with this application, nevertheless, Applicants are providing for the Examiner's review and convenience, a copy of each of the above-cited Caruthers, Gerhard, Manning and Miller documents, attached to this paper as Exhibits 1, 2, 3 and 4, respectively.**

2. **Self-Indicating**

As background to this issue, Applicants point out that former claims in this case recited the language wherein the modified nucleotides or indicator molecules are "self-signalling or self-indicating or self-detecting." In their February 2, 1999 Supplemental Amendment, Applicants deleted the first and second terms from the claim language, leaving the instant "self-indicating" terminology. In their July 24, 1998 Supplemental Response, Applicants had submitted (as Exhibits 1 and 2) two U.S. patents, U.S. 4,981,653 and 4,408,202, that issued with several claims reciting the term "self-indicating." Later, in their aforementioned February 2, 1999 Supplemental Amendment, Applicants submitted four scientific papers which also disclosed the language "self-indicating."

It is believed that the language at hand, "self-indicating," is neither vague or indefinite, but rather is sufficiently clear and definite to pass muster under the second paragraph of §112. Determining whether a claim is definite requires an analysis of "whether one skilled in the art would understand the bounds of the claim when read in light of the specification . . . . If the claims read in light of the specification reasonably apprise those skilled in the art of the scope of the invention, Section 112 demands no more." Miles Lab., Inc. v. Shandon, Inc., 997 F.2d 870, 875, 27 USPQ2d 1123, 1126 (Fed. Cir. 1993). Examined under that well-established legal test, the present claim language is proper and definite, and clearly defines Applicants' invention.

In view of the foregoing remarks, submitted exhibits and the legal standard for claim definiteness, it is believed that the "self-indicating" language in the present claims is altgether proper and clearly and reasonably apprises the reader (a skilled artisan) of the scope of Applicants' invention.

3. **Disruptive Base Labeling Positions**

The issue regarding disruptive base labeling positions was the subject of a first paragraph, §112 rejection in the January 6, 1998 Office Action. In that rejection, it was indicated on page 5 of that Office Action that "the disclosure is enabling only for claims limited to a scope of covalent attachment sites of the cited "Sig" moiety to bases of nucleic acids wherein said sites are either the N^2 of guanine, the N^6 of adenine, the N^4 of cytosine, or the C^6 of uracil. . . . the instant disclosure does not discuss in any way the preparation of N-1 or N-3 modified purines or N-3 or C-2 modified pyrimidines. . ."

It was suggested and discussed briefly at the March 23, 1999 interview that using Applicants' disclosure and armed with the knowledge in the art, the skilled artisan could prepare modified nucleotides labeled in the so-called non-Ward base positions, namely for example, the N-1 and N-3 positions of modified purines and the N-3 and C-2 positions of modified pyrimidines. As a followup to the March 23, 1999 interview, Applicants are submitting various publications listed below as support first, for modifying purines at, among others, the N-1 and N-3 positions, and second, for modifying pyrimidines at, among others, the N-3 and C-2 positions.

N-1 Position in Purines

Montgomery, J. A. and H. Jeanette Thomas, "4-Amino-7-β-D-Ribofuranosyl-7H-Imidazol[4,5-*d*]-v-Triazine(2-Aza-Adenosine) The Synthesis of 2-Azapurine Nucleosides from Purine Nucleosides Accomplished via Ring Opening Followed by Reclosure with Nitrous Acid," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 118, pages 681-685 [copy attached as Exhibit 5];

Shibaev, V. N. and S. M. Spiridonova, "1-Methyladenosine-5'-(α- -Glucopyranosyl Pyrophosphate): Methylation of Adenosine Derivatives," in Synthetic Procedures in Nucleic Acid Chemistry, Volume I, W. Werner Zorbach and R. Stuart Tipson, Editors, Interscience Publishers, New York, 1968, No. 14, pages 461-462 [Exhibit 6].

N-3 Position in Purines

H. Jeanette Thomas and John A. Montgomery, "3-Benzylpurines," in Synthetic Procedures in Nucleic Acid Chemistry, *supra*, No. 10, pages 28-30 [Exhibit 7].

C-2 Position in Purines

Kaneko et al., "8,2'-Anhydrides of Purine -8-Thiol Nucleosides (or of Purine 2'-Thionucleosides): Synthesis of 8,2'-Anhydronucleosides of Purine-8-thiol [or of 8,2'-Anhydro-(2'-thionucleosides)] by use of Diphenyl Carbonate as the Cyclizing Agent," in Nucleic Acid Chemistry, *supra*, No. 103, pages 395-399 [Exhibit 8].

C-4 (keto) Position in Purines

Robins, M. J. and G. L. Bason, "6-Chloro-9-(2-Deoxy-β-D-Erythro-Pentofuranosyl)Purine from the Chlorination of 2'-Deoxyinosine: Direct Replacement of the 6-Oxo Group by a Chlorine Atom in a Purine 2'-Deoxynucleoside; Stabilization of the Glycosyl Bond Towards Cleavage by Acid," in Nucleic Acid Chemistry, *supra*, No. 104, pages 601-606 [Exhibit 9]; and

Zemlicka, J. and J. Owens, "6-Chloro-9-β-D-Ribofuranosylpurine: A Versatile Intermediate in the Synthesis of Purine Ribonucleosides," in Nucleic Acid Chemistry, *supra*, No. 106, pages 611-614 [Exhibit 10].

N-3 Position in Pyrimidines

Kochetkov et al., "3-Methyluridine 5'-Phosphate: Methylation of Uridine Derivatives with Diazomethane; Phosphorylation of Nucleosides with Pyrophosphoryl Chloride," in Synthetic Procedures in Nucleic Acid Chemistry, *supra*, No. 152, pages 497-499 [Exhibit 11]; and

Zemlicka, J., "3-Methyl-6-Azauridine [4-Methyl-2-β-D-Ribofuranosyl-AS-Triazin-3,5-(2H,4H)-Dione]: Alkylation of a Nucleoside Antimetabolite by Use of N,N-Dimethyl-formamide Dimethyl Acetal," in Nucleic Acid Chemistry, *supra*, No. 78, pages 451-453 [Exhibit 12].

C-2 Position in Pyrimidines

Piskala, A. and F. Sorm, "1-β-D-Ribofuranosyl-δ-Triazine-2,4-(1H,3H)-Dione (5-Azauridine): Direct Synthesis of a 5-Azapyrimidine Ribonucleoside by the Fischer-Helferich Procedure," in Nucleic Acid Chemistry, *supra*, No. 79, pages 455-459 [Exhibit 13].

C-6 Position in Pyrimidines

Poverenny et al., "Immunological Approaches to DNA Structure Investigation - I: Immunochemical Identification of the Product of Cytosine Modification with Bisulfite and *O*-Methylhydroxylamine Mixture," Molecular Immunology 16:313-316 (1979) [Exhibit 14];

Visser, D. W. and P. Roy-Burman, "5-Hydroxyuridine 5'-Phosphate Derivatives: Substitution Reactions at the Pyrimidine Ring of Nucleotides," in Synthetic Procedures in Nucleic Acid Chemistry, *supra*, No. 151, pages 493-496 [Exhibit 15]; and

Cadet, J., "1-(2-Deoxy-β-D-Erythro-Pentopyranosyl)Uracil and Its α-D Anomer: Acid-Catalyzed Isomerization of the Glycosyl Group in 5,5-Dibromo-2'-deoxy-5,6-dihydro-6-hydroxyuridine," in Nucleic Acid Chemistry, *supra*, No. 55, pages 311-315 [Exhibit 16].

In light of Applicants' original disclosure and the knowledge in the art at the time their application was originally filed in 1982, it is respectfully submitted that the skilled artisan could have practiced the present invention by modifying nucleotides with the placement of detectable moieties in the so-called "non-Ward" disruptive base positions. Reconsideration and withdrawal of the enablement rejection under §112, first paragraph, is respectfully urged.

4. **Monosaccharide**

Again, as background to this issue, Applicants wish to point out that the original claims in this application were directed to SM being a "sugar moiety." In the January 6, 1998 Office Action, the claims were rejected under 35 U.S.C. §112, first paragraph, "as the disclosure is enabling only for claims limited to "SM" moieties which are either ribose or deoxyribose." In Applicants' July 24, 1998 Supplemental Response to their July 6, 1998 Amendment Under 37 C.F.R. §1.115, the term "sugar moiety" was narrowed to "monosaccharide moiety." In so doing, Applicants offered supporting evidence in their February 2, 1999 Supplemental Amendment to their July 24, 1998 Supplemental Response, citing portions in the specification (page 90, last paragraph; page 93, first paragraph; page 103, second paragraph; and originally filed claims 1, 142 and 143), as well as two art-recognized definitions for the term "monosaccharide" (Stenesh, Dictionary of Biochemistry and Molecular Biology (1989); and Stryer, Biochemistry, 3rd edition, 1988).

At the March 23, 1999 interview, the "monosaccharide" issue was briefly discussed and a counterproposal for the even more narrower term "furanose" was broached but not thoroughly discussed. As indicated in the opening remarks of this paper, all of the claims reciting "SM" do so in the context of a "furanose moiety." As in the case of a "monosaccharide moiety," the term "furanose moiety" is likewise thoroughly supported and

enabled by Applicants' original disclosure. Moreover, this terminology has gained such a widespread acceptance both in the patent and scientific literature, that its use in the present claims is altogether proper.

At the outset, Applicants wish to point out that the term "furanose" is well defined in the literature. Resort may be made to several scientific dictionaries or glossaries, including but not limited to the following:

Glossary of Biochemistry and Molecular Biology, Revised Edition, David M. Glick, Portland Press, London and Miami, 1997, page 69 (Exhibit 17):

> **furanose** The form of a sugar when it is condensed into a five-membered ring. It consists of four carbon atoms and the oxygen atom that is the link to the anomeric carbon atom (*see also* pyranose)

Macmillan Dictionary of Chemistry, D. B. Hibbert and A. M. James, Macmillan Reference Books, London and Basingstoke, 1997, page 204 (Exhibit 18):

> **furanose** Form of a sugar that contains the five-membered ring furan (*see* HETEROCYCLIC COMPOUNDS, structure II) (e.g., ribose, 2-deoxyribose, see PENTOSES), existing in the α- and β-forms. Furanose sugars are less stable than the PYRANOSE forms.

Concise Encyclopedia Biochemistry and Molecular Biology, 3rd Edition, Thomas A. Scott and E. Ian Mercer, Walter de Gruyter, Berlin and New York, 1997, page 93, right column (Exhibit 19):

> . . . If the half-acetal bonds links C1 to C4, the resulting 5-membered ring is a fructose, . . . Most monosaccharides are pyranoses, but the furanose form is present in some oligosaccharides, e.g. arabans. . . The furanose ring is nearly planar, . . .

Oxford Dictionary of Biochemistry and Molecular Biology, A. D. Smith et al. (editors), Oxford University Press, Oxford and New York, 1997, page 249 (copy attached as Exhibit 20):

> **furanose** monosaccharide or monosaccharide derivative whose molecule contains a furanoid ring. *Compare* **pyranose, sephanose.**

Concise Dictionary of Biomedicine and Molecular Biology, Pei-Show Juo, Ph.D., CRC Press, Boca Raton and New York, 1996, page 399 (Exhibit 21):

> **Furanose** A monosaccharide with a five-membered ring structure, *e.g.*, ribose and fructose.

A Dictionary of Chemistry, 3rd Edition, John Daintith, Editor, Oxford University Press, Oxford and New York, 1996, page 217 (Exhibit 22):

furanose A sugar having a five-membered ring containing four carbon atoms and one oxygen atom.

A Dictionary of Biology, 3rd Edition, Oxford University Press, Oxford and New York, 1996, page 213 (Exhibit 23):

furanose A sugar having a five-membered ring containing four carbon atoms and one oxygen atom.

The Facts On File Dictionary of Biotechnology and Genetic Engineering, Mark L. Steinberg, Ph.D., Sharon D. Cosloy, Ph.D. and Edmund H. Immergut, Ph.D. (Series Editor), Facts On File, New York, 1994, page 75 (Exhibit 24):

furanose A ring form of a sugar in which the ring is made up of four carbon atoms and one oxygen. The term designates a large group of sugars that form this type of ring when dissolved in water.

Glossary of Biotechnology Terms, Manfred H. Fleschar, Ph.D. and Kimball R. Nill, Technomic Publishing Co., Inc., Lancaster, PA, 1993, page 57 (Exhibit 25):

Furanose A sugar molecule containing the five-membered furan ring.

Dictionary of Biotechnology, 2nd Edition, James Coombs, Stockton Press, New York, 1992, page 138 (Exhibit 26):

furanose A monosaccharide that has a five-membered ring structure consisting of four carbon and one oxygen atoms.

Glossary of Biochemistry and Molecular Biology, David M. Glick, Ph.D., Raven Press, New York, 1990, page 65 (Exhibit 27):

furanose The form of a sugar when it is condensed into a five-membered ring, consisting of four carbon atoms and the oxygen atom, which is the link to the anomeric carbon atom (*see also* pyranose).

A Concise Dictionary of Chemistry, New Edition, Oxford University Press, Oxford and New York, 1990, page 129 (Exhibit 28):

furanose A sugar having a five-membered ring containing four carbon atoms and one oxygen atom.

Henderson's Dictionary of Biological Terms, 10th Edition, Eleanor Lawrence, John Wiley & Sons, New York, 1989, page 193 (Exhibit 29):

> **furanose** a monosaccharide in the form of a 5-membered ring with 4 carbon atoms and 1 oxygen atoms. *cf.* pyranose.

The Facts On File Dictionary of Biology, Elizabeth Tootill, Editor, Facts On File, Inc., New York, 1981, pages 106 & 254-255 (Exhibit 30):

> **furanose** A sugar that has a five-membered ring (four carbon atoms and one oxygen atom). *See also* sugar.
>
> **sugar** (saccharide) . . . The ring forms of monosaccharides are derived by reaction of the aldehyde or ketone group with one of the carbons at the other end of the chain. It is possible to have a six-membered (*pyranose*) ring or a five-membered (*fructose*) ring. . .

The Facts On File Dictionary of Chemistry, John Daintith, Editor, Facts On File, Inc., New York, 1981, page 89 (Exhibit 31):

> **furanose** A sugar that has a five-membered ring (four carbon atoms and one oxygen atom). *See also* sugar.

Turning to the Applicants' original disclosure, the specification is replete with literal support for the "furanose moiety" language at hand, largely based upon the chemical structures and chemical names found throughout. The following eight pages in the specification contain chemical structures showing the furanose ring for the sugar moiety now being claimed in the present invention:

Page 2 (lower portion of the page)

Page 4 (three instances where the furanose ring is shown)

Page 5 (the polynucleotide structure with three furanose rings shown)

Page 8 (a single furanose ring near the middle of the page)

Page 14 (a single furanose ring depicted)

Page 15 (three furanose ring structures illustrated)

Page 23 (same as page 5 above)

Page 92 (two furanose rings shown in the middle of the page).

Furthermore, the term "furanosyl" is recited in several chemical compound names in the specification. These recitations include the following three compounds (double underlining having been added):

2-deoxy-3,5-di-O-p-toluyl-D-ribofuranosyl chloride (page 62, lines 9 & 10);

4-amino-5 (tetrazol)-5-yl)-7-(B-D-ribofuranosyl) pyrrolo[2,3-d]pyrimidine (page 72, lines 8 & 9 from the bottom of the page); and

4-amino-5-cyano-7-(β-D-2-deoxyfuranosyl) pyrrolo[2,3-d]pyrimidine (page 73, lines 7 & 8 from the bottom of the page).

Not to be overlooked or discounted are the several U.S. patents that have issued over the past decade or so in which issued claims recite "furanose" as an element for a nucleotide or related compound. Illustrative of such patents are the following three:

U.S. Patent No.	Exhibit	Inventor(s)	Exemplary Claims
5,817,638 (10/6/98)	32	Hostetler	Claims 1 & 2 ("wherein the pentose group is a furanose . . .")
5,639,873	33	Barascut et al.	Claims 9-11 ("wherein oxygen is the heteroatom of the furanose ring of the nucleotides of said DNA type oligonucleotide or RNA type oligonucleotide")
5,571,795 (11/5/96)	34	Kahne et al.	Claim 51 ("said glycosyl moiety is a furanose selected from the group consisting of . . .")
5,082,934 (1/21/92)	35	Saba et al.	Claim 4 ("or NH is attached to the 1' portion of the furanose ring . . .")

In view of Applicants' original disclosure cited above, and the art-recognized use of "furanose" both in technical dictionaries and glossaries as described above, and in the aforementioned patent literature, it is respectfully

submitted that the term "furanose moiety" in the present claims is proper and reasonable. Moreover, the use of this term does not extend the scope of Applicants' invention beyond any boundaries permitted under U.S. patent law. Entry of the claim amendments for the "furanose moiety" is respectfully requested.

5. **Fragments Language in Claim 329**

At the March 23, 1999 interview, the Examiner raised an issue regarding the scope of the language presented in claim 329. Prior to the amendments above, claim 329 recited in its first process step "incorporating one or more modified nucleotides or an oligo- or polynucleotide comprising one or more modified nucleotides into a nucleic acid or nucleic acid fragments complementary to said nucleic acid of interest . . ." As indicated in the opening remarks of this paper, the first step of claim 329 now reads "providing labeled nucleic acid fragments, each fragment being complementary to a portion of or to said nucleic acid of interest, wherein each of said fragments comprise one or more modified nucleotides . . ."

In light of the above amendments to claim 329, it is believed that this issue has been obviated.

6. **February 2, 1999 Amendments to Independent Claims**

As a background to this issue, in their February 2, 1999 Supplemental Amendment, Applicants amended claims 284, 331 and 337 by adding or deleting certain language so as to clarify the subject matter being claimed. For example, in the case of claim 284, language was added to the claim. There, in the context of the covalent attachment of the detectable Sig moiety, the phrase "or nucleic acid hybridization" was added in the alternative to "double helix formation," thus making the claim read in three instances:

> . . . such covalent attachment does not substantially interfere with double helix formation or nucleic acid hybridization; . . .

In their February 2, 1999 Supplemental Amendment, Applicants also deleted certain language from claims 331 and 337. In claim 331, which depends from claims 329 and 373, Applicants deleted in three instances the phrase "and such covalent attachment does not substantially interfere with double helix formation." The same amendments were effected to claim 337 which is directed to a process for preparing a labeled oligo- or polynucleotide.

The amendments to claim 284 were discussed in Applicants' February 2, 1999 Supplemental Amendment beginning on page 16 and continuing through the first indented paragraph of page 17. With respect to claims 331 and 337, Applicants explained the reason for those amendments on page 18 of their February 2, 1999 Supplemental Amendment where the following paragraph was offered:

> Claims 331 and 337 are directed to sequencing and preparation processes, respectively. Both claims have been amended by three deletions to the Markush nucleotide members (i, ii and iii). In each of these members, the phrase "and such covalent attachment does not substantially interfere with double helix formation." Because double helical formation is neither an objective of nor a concern in sequencing or preparing the modified nucleotides of the present invention, it is believed that the language of claims 331 and 337 is improved by deleting a characteristic that is not necessary to carry out the sequencing or preparation process.

Thus, rather than attempting to change arbitrarily the scope of their claimed invention, Applicants were clarifying the subject matter. In the case of claim 284, a sincere effort was made to define the covalent attachment in terms of nucleic acid hybridization in a detection process that specifically relies on hybridization between the nucleic acid of interest and the labeled oligo- or polynucleotide. In the case of claims 331 and 337, another sincere effort was made to clarify the subject matter by deleting what could be termed an extraneous characteristic that is not necessary to carry out the sequencing or preparation process of those claims. In some ways, it would be akin to describing a book based in part by the bookshelf in which it has

been placed. or, reviewing a movie based upon the theater in which it was seen.

* * * * * * *

SUMMARY AND CONCLUSIONS

Claims 401-512 have been added above and claims 284, 314-315, 329, 331-332, 337, 348, 373, 376-377, 381, 385 and 390 have been amended.

The fee for new claims 401-512 is believed to be $2,232, based upon the presentation of 124 new additional claims (124 X $18 = $2,232). As indicated in the accompanying Transmittal form, authorization is hereby given to charge the amount of $2,232 to Deposit Account No. 05-1135. If any other fee or fees are deemed necessary in connection with this Fourth Supplemental Amendment, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

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